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L1: Entry 1 of 6

File: USPT

Oct 15, 2002

US-PAT-NO: 6465204

DOCUMENT-IDENTIFIER: US 6465204 B1

TITLE: Amidase

DATE-ISSUED: October 15, 2002

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Murphy; Dennis	Paoli	PA		
Reid; John	Bryn Mawr	PA		
Robertson; Dan	Haddonfield	NJ		

US-CL-CURRENT: 435/18; 435/227, 435/228, 435/230, 435/252.3, 435/320.1, 435/69.1,  
530/350, 536/23.2, 536/23.7

## CLAIMS:

What is claimed is:

1. A method for removal of arginine, phenylalanine or methionine from the N-terminal end of peptides in peptide or peptidomimetic synthesis, comprising: contacting a polypeptide comprising at least 30 consecutive amino acid residues homologous with an enzyme comprising an amino acid sequence which is at least 70% identical to the amino acid sequence set forth in SEQ ID NO:2, which is effective for removal of arginine, phenylalanine or methionine from the N-terminal end of peptides in peptide or peptidomimetic synthesis, wherein the enzyme has amidase activity.

2. The method of claim 1, wherein the enzyme comprises an amino acid sequence which is at least 90% identical to the amino acid sequence set forth in SEQ ID NO:2.

3. The method of claim 1, wherein the enzyme comprises an amino acid sequence which is at least 95% identical to the amino acid sequence set forth in SEQ ID NO:2.

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L1: Entry 2 of 6

File: USPT

Aug 6, 2002

US-PAT-NO: 6429004

DOCUMENT-IDENTIFIER: US 6429004 B1

TITLE: Amidase

DATE-ISSUED: August 6, 2002

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Murphy; Dennis	Paoli	PA		
Reid; John	Bryn Mawr	PA		
Robertson; Dan	Haddonfield	NJ		

US-CL-CURRENT: 435/252.3; 435/227, 435/228, 435/230, 435/320.1, 435/69.1, 530/350,  
536/23.2, 536/23.7

## CLAIMS:

What is claimed is:

1. A method for producing a polypeptide comprising: transforming or transfecting a cell with a vector containing: a) a polynucleotide encoding a polypeptide of SEQ ID NO:2; or b) a polynucleotide which encodes a polypeptide that is at least 70% identical to a polypeptide of SEQ ID NO:2 having amidase activity, such that the cell expresses the polypeptide encoded by the polynucleotide.
2. The method of claim 1, wherein the vector is an expression vector.
3. The method of claim 1, wherein the polynucleotide is set forth in SEQ ID NO:1.
4. The method of claim 2, wherein the vector is a plasmid.
5. The method of claim 2, wherein the vector is virus-derived.
6. The method of claim 1, wherein the cell is a prokaryote.
7. The method of claim 1, wherein the cell is a eukaryote.

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L1: Entry 3 of 6

File: USPT

Oct 24, 2000

US-PAT-NO: 6136583

DOCUMENT-IDENTIFIER: US 6136583 A

TITLE: AMIDASE

DATE-ISSUED: October 24, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Murphy; Dennis	Paoli	PA		
Reid; John	Bryn Mawr	PA		
Robertson; Dan	Haddonfield	NJ		

US-CL-CURRENT: 435/227; 435/228, 435/230, 435/252.3, 435/320.1, 435/69.1, 530/350,  
536/23.2, 536/23.7

## CLAIMS:

What is claimed is:

1. A purified variant protein of SEQ ID NO: 2 which is at least 70% identical, but not 100% identical to SEQ ID NO: 2 and which catalyze removal of arginine, phenylalanine or methionine from the N-terminal end of peptides in peptide or peptidomimetic synthesis.
2. The purified protein of claim 1, wherein the variants are at least about 90% identical to SEQ ID NO:2.
3. The purified protein of claim 1, wherein the variants are at least about 95% identical to SEQ ID NO:2.
4. The purified protein of claim 1, which comprises at least 30 contiguous amino acid residues.
5. A method for removal of arginine, phenylalanine or methionine from the N-terminal end of peptides in peptide or peptidomimetic synthesis, comprising:  
contacting the peptides with an amount of an enzyme of claim 1 effective for removal of arginine, phenylalanine or methionine from the N-terminal end of peptides in peptide or peptidomimetic synthesis.

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L1: Entry 4 of 6

File: USPT

Dec 21, 1999

US-PAT-NO: 6004796

DOCUMENT-IDENTIFIER: US 6004796 A

TITLE: Amidase

DATE-ISSUED: December 21, 1999

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Murphy; Dennis	Paoli	PA		
Reid; John	Bryn Mawr	PA		
Robertson; Dan	Haddonfield	NJ		

US-CL-CURRENT: 435/227; 435/228, 435/230, 435/252.3, 435/320.1, 435/69.1, 530/350,  
536/23.1, 536/23.2, 536/23.7

## CLAIMS:

What is claimed is:

1. An isolated polypeptide having an amino acid sequence as set forth in SEQ ID NO. 2.

2. An isolated polypeptide selected from the group consisting of:

a) an amino acid sequence set forth in SEQ ID NO: 2 or an enzymatically active fragment thereof;

b) a polypeptide comprising at least 30 consecutive amino acid residues homologous with an enzyme of a).

3. An isolated polypeptide selected from the group consisting of:

a) an amino acid sequence set forth in SEQ ID NO: 2 or an enzymatically active fragment thereof;

b) a polypeptide comprising at least 30 consecutive amino acid residues homologous with an enzyme of a), which is effective in removal of arginine, phenylalanine or methionine from the N-terminal end of peptides in peptide or peptidomimetic synthesis.

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L1: Entry 5 of 6

File: USPT

Nov 16, 1999

US-PAT-NO: 5985646

DOCUMENT-IDENTIFIER: US 5985646 A

\*\* See image for Certificate of Correction \*\*

TITLE: Amidase

DATE-ISSUED: November 16, 1999

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Murphy; Dennis	Paoli	PA		
Reid; John	Bryn Mawr	PA		
Robertson; Dan	Haddonfield	NJ		

US-CL-CURRENT: 435/252.3; 435/227, 435/228, 435/320.1, 536/23.2, 536/23.7

## CLAIMS:

What is claimed is:

1. An isolated polynucleotide `encoding an enzyme with amidase activity and which is` selected from the group consisting of:

fragments of SEQ ID NO:1 that are at least 35 bases in length and that hybridize to a nucleic acid sequence encoding the polypeptide set forth in SEQ ID NO:2 under conditions that include 0.9 M NaCl, 5.0 mM NaH<sub>2</sub>PO<sub>4</sub>, 5.0 mM Na<sub>2</sub>EDTA, 0.5% SDS and 10.times. Denhardt's at about 45.degree. C.

2. A polynucleotide of claim 1 wherein the polynucleotide is DNA.
3. A polynucleotide of claim 1 wherein the polynucleotide is RNA.
4. A vector comprising the polynucleotide of claim 1.
5. A host cell comprising a vector of claim 4.

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## End of Result Set

  

L1: Entry 6 of 6

File: USPT

Mar 2, 1999

US-PAT-NO: 5877001

DOCUMENT-IDENTIFIER: US 5877001 A

\*\* See image for Certificate of Correction \*\*

TITLE: Amidase

DATE-ISSUED: March 2, 1999

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Murphy; Dennis	Paoli	PA		
Reid; John	Bryn Mawr	PA		
Robertson; Dan	Haddonfield	NJ		

US-CL-CURRENT: 435/227; 435/228, 435/230, 435/252.3, 435/320.1, 435/69.1, 530/350,  
536/23.1, 536/23.2, 536/23.7

## CLAIMS:

What is claimed is:

1. An isolated polynucleotide sequence encoding a polypeptide of SEQ ID NO:2.
2. An isolated polynucleotide selected from the group consisting of:
  - a) SEQ ID NO:1;
  - b) SEQ ID NO:1 wherein T can also be U; and
  - c) nucleic acid sequences complementary to a) and b).
3. The polynucleotide of claim 1, wherein the polynucleotide is isolated from a prokaryote.
4. An expression vector including the polynucleotide of claim 1.
5. The vector of claim 4, wherein the vector is a plasmid.
6. The vector of claim 4, wherein the vector is virus-derived.
7. A host cell stably transformed with the vector of claim 4.
8. The host cell of claim 7, wherein the cell is a prokaryotic cell.
9. The host cell of claim 7, wherein the cell is a eukaryotic cell.
10. A method for producing a polypeptide comprising:
  - a) culturing the host cells of claim 7;
  - b) expressing from the host cell of claim 7 a polypeptide encoded by said DNA;

and

c) isolating the polypeptide.

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L2: Entry 1 of 2

File: USPT

Feb 25, 2003

US-PAT-NO: 6525190

DOCUMENT-IDENTIFIER: US 6525190 B1

TITLE: Amidase

DATE-ISSUED: February 25, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Murphy; Dennis	Malvern	PA		
Reid; John	Ardmore	PA		
Robertson; Dan	Solana Beach	CA		

US-CL-CURRENT: 536/24.32; 435/227, 435/228, 435/230, 435/6, 536/23.2, 536/23.7

## CLAIMS:

We claim:

1. A nucleic acid probe comprising an oligonucleotide having at least 70% identity to a nucleic acid region of a nucleic acid encoding the amino acid sequence of SEQ ID NO:2, or its full complement and which forms a detectable target:probe duplex with a nucleic acid that encodes a polypeptide having amidase activity, or its full complement, under hybridization conditions comprising 0.9 M NaCl, 50 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 7.0, 5.0 mM Na<sub>2</sub>EDTA, 0.5% SDS, 10.times.Denhardt's, and 0.5 mg/mL polyriboadenylic acid at 45.degree. C.
2. The probe of claim 1, wherein the oligonucleotide is DNA.
3. The probe of claim 1, wherein the oligonucleotide is at least 10 contiguous bases in length.
4. The probe of claim 1, wherein the probe further comprises a detectable label.
5. The probe of claim 4, wherein the probe comprises a detectable label selected from a radioactive label, a fluorescent dye or an enzyme.
6. The probe of claim 1, wherein the oligonucleotide has at least 90% identity to a nucleic acid region of a nucleic acid encoding the amino acid sequence of SEQ ID NO:2, or its full complement.
7. The probe of claim 1, wherein the oligonucleotide has at least 95% identity to a nucleic acid region of a nucleic acid encoding the amino acid sequence of SEQ ID NO:2, or its full complement.
8. The probe of claim 1, wherein the oligonucleotide has at least 100% identity to a nucleic acid region of a nucleic acid encoding the amino acid sequence of SEQ ID NO:2, or its full complement.
9. The probe of claim 1, wherein the oligonucleotide is at least 15 contiguous bases in length.

10. The probe of claim 1, wherein the oligonucleotide is at least 30 contiguous bases in length.

11. The probe of claim 1, wherein the oligonucleotide is at least 50 contiguous bases in length.

12. The nucleic acid probe of claim 1, wherein the target:probe duplex is maintained under wash conditions including 1.times.SET (150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na<sub>2</sub>EDTA) containing 0.5% SDS at room temperature.

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## End of Result Set

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L2: Entry 2 of 2

File: USPT

Dec 31, 2002

US-PAT-NO: 6500659

DOCUMENT-IDENTIFIER: US 6500659 B1

TITLE: Amidase

DATE-ISSUED: December 31, 2002

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Murphy; Dennis	Paoli	PA		
Reid; John	Bryn Mawr	PA		
Robertson; Dan	Haddonfield	NJ		

US-CL-CURRENT: 435/227, 435/228, 435/230, 435/252.3, 435/320.1, 435/69.1, 530/350,  
536/23.2, 536/23.7

## CLAIMS:

What is claimed is:

1. A purified polypeptide selected from the group consisting of: a) a polypeptide comprising an amino acid sequence which is at least 70% identical to an amino acid sequence as set forth in SEQ ID NO:2; and b) a polypeptide comprising at least 30 consecutive amino acid residues homologous with the polypeptide of a); wherein the polypeptides of a) and b) have amidase activities.
2. The purified polypeptide of claim 1, wherein the polypeptide is effective in the enzymatic removal of arginine, phenylalanine or methionine from the N-terminal end of peptides in peptide or peptidomimetic synthesis.
3. The purified polypeptide of claim 1, wherein the polypeptide is from a thermophilic bacteria.
4. The purified polypeptide of claim 1, wherein the polypeptide is a thermostable enzyme that catalyzes the removal of arginine, phenylalanine, or methionine from the N-terminal end of peptides in peptide or peptidomimetic synthesis.
5. The purified polypeptide of claim 1, wherein the polypeptide has a molecular weight of about 68.5 kilodaltons.
6. The purified polypeptide of claim 1, wherein the polypeptide is at least 90% but not 100% identical to an amino acid sequence as set forth in SEQ ID NO:2.
7. A purified peptide comprising least 30 consecutive amino acid residues homologous with the polypeptide of claim 6.
8. A purified polypeptide characterized as catalyzing the removal of arginine, phenylalanine, or methionine from the N-terminal end of peptides and having a sequence as set forth in SEQ ID NO:2 with one or more conservative amino acid substitutions.

9. A fusion enzyme construct comprising a sequence as set forth in SEQ ID NO:2 and a leader or secretory sequence, wherein the fusion polypeptide has amidase activity.
10. The fusion enzyme construct of claim 9, further comprising a promoter sequence operably linked to the construct.
11. The fusion enzyme construct of claim 10, wherein the promoter is a bacterial promoter.
12. The fusion enzyme construct of claim 11, wherein the promoter is lacI, lacZ, T3, T7, gpt, lambda PR, PL or trp.
13. The fusion enzyme construct of claim 10, wherein the promoter is a eukaryotic promoter.
14. The fusion enzyme construct of claim 13, wherein the promoter is a CMV promoter, HSV thymidine kinase, early SV40, late SV40, retroviral LTR or metallothionein promoter.
15. The fusion enzyme construct of claim 10, wherein the construct is in a host cell.
16. The fusion enzyme construct of claim 15, wherein the host cell is a bacterial cell.
17. The fusion enzyme construct of claim 15, wherein the host cell is a eukaryotic cell.
18. The fusion enzyme construct of claim 16, wherein the host cell is E. coli, Bacillus subtilis, Salmonella typhimurium, Pseudomonas sp., Streptomyces sp. or Staphylococcus sp.
19. The fusion enzyme construct of claim 17, wherein the host cell is a C127, 3T3, CHO, HeLa or a BHK cell.
20. The purified polypeptide of claim 1, wherein the polypeptide is at least 95% identical to an amino acid sequence as set forth in SEQ ID NO:2.
21. The polypeptide of claim 20, wherein the polypeptide has amidase activity.
22. The polypeptide of claim 21, wherein the polypeptide is a thermostable enzyme that catalyzes the removal of arginine, phenylalanine, or methionine from the N-terminal end of peptides.
23. The polypeptide of claim 6, wherein the polypeptide has amidase activity.
24. The polypeptide of claim 23, wherein the polypeptide is a thermostable enzyme that catalyzes the removal of arginine, phenylalanine, or methionine from the N-terminal end of peptides.